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A STUDY OF RECIPROCAL CROSSES.

This Bulletin contains the results of studies upon the cross-fertilization of certain varieties of tomatoes and squashes.

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FERTILIZATION PROBLEMS: A STUDY OF RECIPROCAL CROSSES.

M. B. Cummings.

In attempting to account for failure in the making of reciprocal crosses one faces a perplexing problem. The number of cases in which reciprocal crosses cannot be made is small, when compared with that of successful ones. It is not the number of successes or failures, however, which gives the subject prominence. It is not the fact of failure in one case and success in another; but rather the reason for such failure. If we can cross plant A with plant B, why can we not cross plant B with plant A? What is the immediate cause for the refusal of certain plants to cross reciprocally, and how does their refusal manifest itself? To find a satisfactory answer to these questions is the purpose of this investigation.

I. Records of Previous Work.

SOME IMPOSSIBLE RECIPROCAL CROSSES.

No exhaustive list of supposed impossible reciprocal crosses is attempted at this time, since a few cases will serve the purpose of presenting the problem. Kölreuter[1], during a period of eight years, made repeated trials to secure reciprocal crosses with *Mirabilis jalapa* and *Mirabilis longiflora.* He tried more than two hundred times to fertilize *Mirabilis longiflora* by applying pollen of *Mirabilis jalapa,* but without success. The pollen of the former when applied to the stigma of the latter, however, produced fertile seeds. The same difficulty is met with when an

1. Cited by Webber and Swingle. Hybrids and Their Utilization in Plant-Breeding, Yearbook Dept. Agr., 1897, p. 383.

Note. This paper is part of an investigation which has been in progress for the past 12 years by Professor W. M. Munson, Horticulturist to the Station, upon the effects of pollination. Beginning with 1892, occasional reports of progress have appeared in the bulletins and reports of this Station. The studies here reported upon were conducted under Professor Munson's direction.

attempt is made to reciprocally cross *Matthiola annua* and *Matthiola glabra,* two closely related species.[1]

Thuret[3] is authority for the statement that *Fucus vesiculosus* crossed by *Fucus serratus* is fertile, but that reciprocal crosses with these plants cannot be made. Strasburger[2] found that *Orchis fusca* pollen will stimulate the ovules of *Orchis Morio* into normal activity, while pollen of *Orchis Morio* will not form tubes on the stigma of *Orchis fusca.*

Among more familiar plants, other supposed impossible reciprocals are the red currant and common garden tomato, and summer crookneck and bush scallop squashes. These cases will be discussed in detail later in this paper.

PROCESSES LEADING TO THE FORMATION OF SEED.

In order to pave the way for a study of abnormal conditions, it will be well at this point to review in a general way the normal process of fertilization.[3]

It is now well known that the pollen grain, after being lodged on the sticky surface of the stigma, undergoes a process similar to that of the germination of spores of the lower orders of plants, and not greatly unlike that of the germination of seeds. Soon after the deposit of the pollen, the grains absorb moisture and increase in size; the outer wall, the extine, bursts, and inhibition of watery solutions incites to activity the protoplasm of the grain, and movements of cytoplasm within cause the extension of the inner wall so as to form a tube, the so-called pollen tube. Now the pollen tube, once started in its downward course, makes its way through or between the parenchyma cells of the pistil, manifesting its growth by the formation of new nuclei in the protoplasm, and in the extension of the wall in the form of a tube. This tube penetrates to the region of the ovules and when the micropyle is reached the pollen nuclei pass into the embryo-sac, and a union of the elements is effected which results in the formation of seed. Germination when once started may be completed in a few hours, as in the case of *Cereus grandiflorus;* or it may require several months, as in the case with orchids; or almost a year, as is true with the pines.

1. Cited by A. R. Wallace, Darwinism, p. 155.
2. Cited by H. P. Gould, Potency of Pollen, Cornell University Thesis, 1897, p. 28.
3. Cf. also, Rpt. Maine Agr. Expt. Sta., 1898, 219, et seq.

Just how the pollen tube is nourished, how it is guided to the ovarian region, and what finally brings the elements together, is still very largely a matter of theory and conjecture. However, imperfect as our knowledge is concerning these points, numerous observations on various stages and types of germination, enable one to make a few very general remarks on the above questions.

As regards the nourishment of the tubes, it is known that there are two probable sources of food. The pollen grains contain some elements for nourishment, and the conductive tissue is not entirely without food. From chemical analyses it has been shown that pollen cells contain a certain amount of nutritive material such as starch and maltose, in connection with some invertase and diastase to render them available for the nutrition of the pollen tubes. On the part of the pistil and its conductive tissue there is an abundant supply of elaborated food materials such as starch, sugar, and maltose, which, without doubt, are of much value in nourishing the tubes in their passage to the ovules.

It is very probable that the development and action of enzymes facilitate the descent of the pollen tube, and the discovery of a cytolytic enzyme by Marshall Ward[1] in a species of Botrytis, throws further light on the problem of the penetration of tubes.

An analysis of the factors which facilitate the descent of pollen tubes and finally enable them to reach the ovules shows them to be of at least two sorts. First, mechanical contrivances; and secondly, stimulating influences or attractive substances which cause the union of the elements. The mechanical features include the nature of the conductive tissue which, as we know, is a system of thin walled cells through which the pollen tubes pass, growing as they naturally would in the lines of least resistance. Other features of a mechanical nature are also useful; such as papillæ, which are common in the pistils of Cucurbitaceae; and the position of the funiculus and the ovule itself.

The second set of factors, the stimulating or attractive substances, do not admit of as easy demonstration. As a matter of fact, just what actually brings the male and famale nuclei into contact is not known. However, a few probable suppositions may be mentioned here and left for further discussion and investigation.

1. H. Marshall Ward, Annals of Botany, II, 1888.

During the period of receptivity of the pistil and descent of the pollen tube, deep seated changes are going on in the ovary which prepare the ovules for the fertilizing process. This preparation consists in the formation of an organ in the nucellus of the ovule called the embryo-sac, an exceedingly important structure during fecundation. This embryo-sac consists of several parts, which may be referred to as follows: At the micropylar end of the sac is the egg apparatus, which consists of two cells, the synergidae, and another the germ cell or oösphere, which, after fertilization, becomes the embryo. At the opposite end of the sac there are three cells, the antipodals, which are of lesser importance. But the egg apparatus is the dynamic center; and it is supposed that this structure exerts a stimulating influence as the male nucleus nears its destination, the supposition being that a fluid escapes from the synergidae which attracts the pollen spore and unites the elements.

In studying the approach of sexual organs Pfeffer[1] has demonstrated that the spermatozoids of ferns are enticed into the necks of the archegonia by means of malic acid; and the archegonia of mosses attract the spermatozoids by a solution of cane sugar. Before leaving this point, reference should be made to the statement of Strasburger[2], who would explain the fusion of the male and female elements as due to chemitactic and chemotropic influences; the explanation being that the nature and strength of solutions in the pistil and in the vicinity of the ovules attract or repel the pollen tubes.

DETERMINING FACTORS, IN CASES OF ABNORMAL FERTILIZATION.

The nature of the problem under consideration necessitates the study of certain factors which may influence in some way the making of the crosses. The factors here considered are:

A. Incomplete development of pollen tubes, due to
   (1.) Impotent pollen or poisonous stigmatic fluid, or
   (2.) Lack of nourishment of pollen tubes.
B. Non-fusion of nuclei.

1. Cited by Wilson. The Cell in Development and Inheritance, p. 197.
2. Strasburger, Text-book of Botany, pp. 263, 281.

A. Incomplete Development of Pollen Tubes.

(1.) Impotent pollen or poisonous stigmatic fluid.

The study of this feature of the problem had its origin mainly with the investigations of Darwin[1] and no systematic study of self-sterility was taken up till after the publication of Origin of Species in 1859. While horticulturists and other investigators were studying the causes of sterility for many years, it was not till quite recently that the histological study of sterility was begun. Even though the general causes of sterility have been pretty well worked out, yet the details of the problem,—the non-development or incomplete development of the pollen tubes,—is, so far as the writer's knowledge is concerned, still unexplained.

It is well at this point to refer to the investigations of the pioneers on this subject. Darwin, for example, found that in the case of legitimate and illegitimate union of the elements in *Linum perenne,* if pollen of either form be placed upon its own stigmas, the pollen grains would germinate and the tubes enter the tissue of the pistil, but to what extent is not known. Darwin observed here that the impotency of the pollen must be due either to the tubes not reaching the ovules, or to their improper action upon reaching them.

Again, in the case of *Linum grandiflora* where other unions, legitimate and illegitimate were made, it was ascertained by examining the pistils that the pollen of illegitimate unions germinated very rarely, and in those where germination did take place the tubes were short and penetrated the tissue of the pistil only a short distance.

In other cases the pollen seems to be absolutely impotent, and in such cases the trouble seems to lie in the inability of the stigmatic secretion to properly excite the pollen in such a way as to form tubes. This has been found to be the case with some of the Orchidaceae where self pollination results in the death of the flower. Fritz Müller[2] says that pollen masses and the stigma of the same plant in various species of Orchidaceae have actually a deadly effect upon one another, as in the case of *Oncidium oscrohilum.* This was shown by the surface of the stigma in contact with the pollen, and by the pollen itself becoming dark and

1. Darwin. Different Forms of Flowers on Plants of the Same Species. Chap. 1, 2, 3.

2. Fritz Müller, Bot. Ztg., 1868, p. 114.

decaying in from three to five days after pollination. Again with *Oncidium flexuosum,* the plant's own pollen and that of a distinct species were placed side by side, and after five days the latter was perfectly fresh, while the plant's own pollen was brown. These observations are remarkable, for they show that the plant's own pollen not only fails to impregnate the flower, but acts on the stigma, and is acted on in an injurious or poisonous manner. Such action seems to be mutually poisonous.

In 1897 Gould[1] confirmed the work of the older investigators. At the conclusion of his thesis he says: "The power of pollen to produce fecundation exists in every possible degree from perfect potency to absolute impotency. Pollen, like seeds, may be so low in degree of vitality as to be unable to germinate, or be so retarded in its germination that the elements do not come together till the period of receptivity is past. External conditions such as temperature and moisture may influence the vitality of pollen and its potency."

Some recent work by Booth[2] concerning the self-sterility of grapes throws further light on the inactivity of pollen. Results of experiments carried on for several years are summarized in this manner: Poor pollen may be known by a microscopical study of the structure of the grains; the infertile grains are irregular in shape and have sharp angles,—also by the arrangement of pollen, either dry or in fluid media. Fertile pollen comes together in clusters by means of its mucilaginous coatings. Sterile pollen does not have this coating, and comes together only by chance. Culture experiments showed the self-sterile pollen to be lacking in viability, for in most cases such pollen either failed to germinate or gave a low percentage of germination. Such pollen was impotent on pistils of self-sterile varieties, as well as on its own stigmas. Booth explains the reason for this condition of things, as a probable indication that the flowers of the grape are in an evolutionary stage, and are passing from hermaphrodite to staminate and pistillate forms. Mention of these cases is made here to show how infertility is expressed, for the problem of impossible reciprocates bears hard on the subject of sterilty.

(2.) Lack of nourishment of pollen tubes.

1. H. P. Gould, Potency of Pollen, Cornell University Thesis, 1897.
2. N. O. Booth, N. Y. Agr. Exp. Sta. Bul. 224. A Study of Grape Pollen.

It is generally conceded that the descent of the pollen tube is largely dependant on the nature of the stigmatic fluid as a medium for the germination of pollen. During the descent of the tubes certain enzymes are developed; and while the exact nature and importance of these is not worked out, it is thought that they exert a considerable influence on the growth of pollen tubes. If this influence is correct, it follows that unless these nutrients are such as to develop proper conditions for germination, the penetration by pollen tubes cannot be effected. To what extent this will account for failure in making reciprocal crosses is still an open question. It is difficult to understand why trouble should occur in the nourishment of the germ tubes only when certain pollen is used, and not in all cases, and yet a slight disturbance of the sexual organs may be of considerable importance in this connection.

B. Non-fusion of Nuclei.

Whatever reasons there may be for failure in fertilization, it is certain that fecundation cannot be effected unless there is a fusion of male and female nuclei. The fusion of the nuclei takes place only when the pollen tubes develop in such a way as to allow the male nucleus to pass into the öosphere. Pollination and development of pollen tubes may take place and yet fertilization fail to occur. A few instances from other investigations will bear out this statement. Mr. John Scott[1] observed that in the case of *Oncidium sphacelatum* self pollination did not produce capsules, although the stigmas were penetrated by pollen tubes.

Furthermore, in another species, *Oncidium microhilum*, the pollen was good, for with it he fertilized two distinct species; he found its ovules good, for they could be fertilized with another plant of the same species, but this species could not be fertilized with its own pollen, although the pollen tubes penetrated the stigma.

In 1900 Fletcher[2] noted that self-fertile varieties of plums —miner and wild goose—were infertile on each other. In the case of the wild goose plum the pollen germinates, and the tubes pass down to the ovules, but for some reason the two sexes fail

1. Scott, Journal of Proc. Linn. Soc., Vol. III.
2. S. W. Fletcher, Cornell Univ. Exp. Sta., Bul. 181.

to unite. Gould[1] reported a similar case where crookneck squash was pollinated with bush scallop. There was a vigorous development of pollen tubes, since they passed in the vicinity of the ovules, but there was no fusion of the nuclei.

SUMMARY.

In summarizing those cases in which reciprocal crosses have failed, we may note that in some cases pollen tubes do not develop, in others there is a partial germination, and in others still there is a vigorous development of tubes; but in none of these cases is there fusion of the nuclei.

II. Studies by the Author.

A. Red currant and yellow plum tomato.

The difficulty which has been experienced in making reciprocal crosses with red currant tomato, *Lycopersicum pimpinellifolium,* and varieties of *Lycopersicum esculentum,* has given rise to the notion that such reciprocals cannot be made. Wishing to know the facts in the case, the writer began investigations with the following points in view. First, to see if reciprocal crosses with these plants can be made. And secondly, if this is impossible, to find out what constitutes the difficulty.

Plants for this work were grown in the University greenhouse. Seeds of yellow plum and red currant tomatoes were planted in flats, and as the seedlings attained proper size they were transplanted, some into large pots and others into large boxes. As soon as the blossoms appeared, a study of the sexual elements was begun. For the first few weeks following the appearance of the flowers, much time was spent in a study of the pollen of these plants. The results of this investigation are given below.

(1.) A comparison of pollen.

In attempting to account for failure in crosses, one is apt to consider pollen as the source of trouble. In view of this fact, a structural and germinative study of pollen was made. Pollen from flowers of the kinds mentioned above was examined in dry and fluid mounts, but no differences in form or structure could be detected. Although the pollen from both species was to all

1. H. P. Gould, Studies in Potency of Pollen, Cornell Univ. Thesis, 1897.

appearances precisely alike, germinative tests were made to note any differences in rapidity and per cent of germination. For this study sugar solutions of 1, 2, 3, 5, and 10 per cent were taken as culture media. It seems remarkable that plants which are so different in gross structure should show absolutely no difference in structure of their pollen, or in per cent and rapidity of germination. In all cultures, however, the protoplasm streamed out in much the same manner, and measurements were made of the length of pollen tubes, but even here no difference could be detected.

A study of pollen of yellow plum was furthered by making close-pollinations, without emasculating, and covering the flowers to prevent access of foreign pollen. In nearly every case the pollen did its work, for the fruit set and came to maturity. Treated in a similar way flowers of red currant gave similar results.

(2.) Trouble with pistils.

In view of the foregoing study and its results, the writer was convinced that the trouble lay, not with the pollen, but probably with the pistils, or in combining yellow plum pollen and red currant pistils. By this time the process of emasculation was begun, and here great difficulty was experienced. It seemed almost impossible to emasculate the red currant without destroying the upper portion of the pistil. This trouble led to a comparative study of the pistils of red currant and yellow plum. This comparison has revealed the major reason for inability to cross the plants.

The pistil of the red currant is considerably smaller than the yellow plum, as can be seen with only a superficial examination; but a more careful study reveals the fact of minor differences which are of considerable importance. The most striking difference, aside from that of size, is to be found at the junction of style and ovary. At this point, in the case of red currant, there is a constriction in the pistil, which is much narrower and is placed in a slight depression in the ovary. A critical examination, however, shows no joint, although there is a slight dissimilarity in size and structure of cells. The style of the red currant is, then, largest half way between the ovary and stigma, that is, it tapers toward the stigma and also toward the ovary.

The pistil of the yellow plum, on the other hand, is continuous with the tissue of the ovary and does not exhibit that narrowed structure which is seen in the red currant; but, in fact, is larger and broader there than elsewhere.

This comparison brings up the mechanical feature in crossing these two species. Here, then, one may note that the red currant flowers can be successfully emasculated only by exercising great care, on account of the ease with which the style separates from the ovary, a slight push to one side being sufficient to detach it. Furthermore the pistil is very slender and exceedingly delicate, and being deprived of the protection which the united stamens afford, it is exposed to changes of temperature and moisture which in most cases are fatal to the pistil.

In successful emasculation of red currant, and with the ordinary method of covering the flowers with paper bags, it was practically impossible to keep the pistils fresh till the period of receptivity. In the majority of cases the pistils withered in the course of twenty-four hours after emasculation, and hardly a single one could be found in perfect condition three days after the removal of stamens. Even when the pistils did survive and were pollinated they did not remain fresh long enough for the pollen to do its work.

In view of these difficulties, it became evident that some means should be provided to prevent the withering and dying out of the pistils. Instead of covering each cluster of flowers with manilla bags, glass bell-jars which covered the whole plant were used, thus eliminating to a marked degree the exposure of the pistils, and securing a fairly uniform condition of moisture and temperature. It should be said here that this scheme was feasible only by using small plants and very large glass bell-jars. By thus protecting the flowers, and in fact the whole plant, less difficulty was experienced in keeping pistils in good condition as long as seemed necessary. Nearly as good results were obtained by using double paper bags, and similarly covering the whole plant. Such good results followed these special methods that the real difficulty in making these reciprocal crosses was considered to be mechanical, and this difficulty can be very largely overcome as previously indicated.

Seeds saved from fruits of these reciprocal crosses, produced fertile plants, and thus we have an answer to our opening ques-

tions: Reciprocal crosses of red currant and yellow plum can be made; but special care must be exercised in emasculating the flowers of red currant, and the pistils must be protected from injurious effects of changes of temperature and moisture. The real difficulty in making these reciprocal crosses is a mechanical one, and lies in the delicate structure of the pistil of the red currant.

B. A STUDY OF SQUASHES.

All the previously mentioned impossible reciprocals are those between distinct species; but with the squashes we have more closely related plants, the summer crookneck and golden custard being horticultural varieties of the same species, *Cucurbita Pepo*, a condition which makes the problem all the more remarkable and adds greatly to the interest of the inquiry for facts in the general problem of impossible reciprocals.

(1.) The field work.

The time when this work was done, and the conditions under which the material was obtained, lend latitude to the problem. Some of the plants for this study were grown in the University greenhouse in the winter and spring of 1903, but most of the material was obtained from plants grown in the garden under normal conditions of culture. Plants which were grown out-of-doors were planted June 18 and began to blossom August 10. Staminate flowers were the first to appear, and at this time a study of the pollen of the two varieties was taken up, which will be described in the histological part of this subject.

Pollinating the Plants—The flowers of the squashes are of exceedingly short duration; the corolla remains open only one day, and generally somewhat less than twenty-four hours. Such short lived flowers necessitated daily attendance in the squash plot in order to do the pollinating when the staminate and pistillate flowers were in prime condition for the operation. Note should be made here of the great care that was taken in securing pure fresh pollen, and applying it to the pistils that were at their height in the period of receptivity. Since the flowers exist only for a short time, pure pollen was easily secured by tying a string over the tip of the corolla the day before the flower opened, thus preventing access and mixture of pollen. Pistillate flowers were

protected in the same way—the stigmas were never exposed except for the moment when pollen was applied to them.

Other methods of protecting the flowers were tried, such as covering with paper bags and glass bell-jars, but were found less satisfactory owing to the fact that a close atmosphere for the entire flower seemed to hasten decay of the pistils, thus shortening the age of the flower and giving less time for the action of pollen. No pollen was used that was more than twenty-four hours old; and all pistils were discarded, which, because of age or structure, were unfit for pollination work.

By actual count, 284 pistils of crookneck squash were pollinated with golden custard. Of this number about one-third were picked off at various stages, and preserved for microscopical study. The rest were left on the vines as long as they remained intact. As would be expected in any case of artificial pollination, a large number of the crosses were a complete failure, as was evidenced by the early death of all parts of the flower. In a few cases, however, the ovary made an unusual growth, due, it was thought, to the scarcity of blossoms, and to the vigor of the plants; and as interesting results were in sight, these fruits were left till frost killed the vines.

No apparent difficulty was experienced in crossing golden custard with crookneck; and pistils of each could be fertilized with pollen of its own kind and from the same plants, but pollen of golden custard would not act on pistils of crookneck. Facts of this nature seemed to indicate that the difficulty lay, not with poor pollen in either case, nor with poor pistils, but rather in the way they were combined.

(2.) Histological studies.

This feature of the work was begun by a study of dry pollen, pollen in fluid mounts, and germinating pollen grains. In this work all study was comparative. Pollen of crookneck and golden custard was taken to the laboratory and examined with high and low power objectives, with grains in dry and fluid mounts; but in size, shape and surface markings the pollen of the two varieties was precisely alike. Pollen grains of the squashes do not germinate readily in artificial media, and because of this fact a study of germinating pollen was attended with poor success. A careful examination of the male elements of the squashes adds nothing to the solution of the problem.

Pistils of crookneck pollinated with golden custard, representing all possible stages, were picked; beginning with those of 24 hours' duration and passing up to those which had been pollinated 7 and 8 days. Pistils generally remain fresh 3 to 4 days, and in some cases they did not show decay till the fifth day after pollination. Of self pollinated and cross pollinated flowers there seemed to be no difference in duration of flowers. In many cases pistils were left on the plant till the stigma and style had decayed and fallen off; in such cases only a portion of tissue, that immediately surrounding the ovules, could be preserved, but this was actually necessary in order to give pollen tubes—if they did germinate and enter the ovarian region—a chance to act, and furthermore to give the investigator an opportunity to study the final action of such tubes.

Of course a study of the germination of pollen tubes necessitated a large amount of histological work, and in order to get material in condition for this study the following method was followed: Pistils were picked, trimmed so as to avoid all unnecessary tissue, killed, and fixed in a one per cent solution of chromo-acetic acid for 30 hours, washed an equal length of time in tap water, hardened and dehydrated in alcohol of 35, 50, 70, 95 per cent solution, and finally in absolute alcohol. The alcohol was replaced by xylol, and the pistils were then imbedded in paraffin. Sections were cut at thicknesses varying from three and one-third to twenty micro-millimeters; twenty micro-millimeters proved the most satisfactory. The three-color-stain—Flemming's safranin, gentian-violet, and orange G. was used. The tissues were mounted in balsam and finally studied with the microscope.

The object of making and examining these sections of the pistils and ovaries was to ascertain if possible, some reason why fecundation failed to occur, the chief aim being to trace the pollen tubes, if formed, from stigma to ovules. The tracing of pollen tubes is a comparatively easy thing to do, provided the tissue is properly handled and successfully stained. The triple stain previously mentioned proved especially good for this work. In this case the cytoplasm of embryo-sac and pollen tube stains from gray to orange, the nucleus violet, and nucleolus red. Besides being different in color, the pollen tubes are distinguished by the peculiar granular structure of their protoplasm.

The germination of golden custard pollen on pistils of crookneck, is not a rapid process. On an average the tubes have to pass through one and three-eighths inches of tissue, and it generally takes about 8 days for the generative nucleus to reach the embryo-sac. In other words the tubes descend at the rate of four and one-fourth millimeters per day. Germination of pollen begins very soon after the deposit of the grains on the stigma, for at the end of 24 hours many of the tubes have attained a length equal to twice and in some cases three times the diameter of the grain. Before the end of the second day the vegetative nucleus has passed into the tube, and soon after this the generative nucleus has also started on its downward course. During the two following days the tubes continue to descend, and oftentimes they branch to some extent. From this time on nothing important happens till the male protoplast reaches the ovarian region and comes in the vicinity of the micropyle. This occurs about the sixth day, or sometimes between the fifth and seventh days. Before the end of the eighth day the generative nucleus has passed the vegetative, enters the embryo-sac, and finds a resting place near the germ cell. This seems to be the final action of the pollen spore, for tissue nearly ten days old shows the same condition as that which has been pollinated eight days. This seems to give a key to the situation. Pollen grains germinate, pollen tubes descend, the pollen spore enters the embryo-sac; but the nuclei do not fuse. Fertilization does not occur. The reason for failure is, apparently, a refusal of the embryo of crookneck to be impregnated by pollen spores of the other variety.

It now becomes evident that where there is no incompatability in the structure of the flowers, where no mechanical difficulties exist, and when the pollen tubes descend and enter the ovarian region, the reason that certain reciprocal crosses cannot be made, is non-fusion of male and female nuclei. The elements do not come together, although the protoplast of the pollen grain arrives, that is, in many cases it enters the embryo-sac.

In some cases the nuclei are some distance apart in the embryo-sac while in others the distance which separates them is only twice their diameter. Such cases are quite common. Now, with these facts before us, we are confronted with the question

as to which element, male or female, refuses to fuse with the other. In other words, does the pollen spore fail to take, or does the germ cell fail to receive? From the nature of the case we suppose that the germ cell will not receive; for the male nucleus has arrived, and therefore, has seemed to have done its work. That the failure to fuse is not due to a difference in structure of the nuclei, is shown by the microscope. The nuclei, so far as the microscope reveals, are of precisely the same size and shape. So that the dissimilarity or differentiation of gametes, if such exist, must be sought in characters which are probably constitutional, perhaps chemical, in nature.

It has been suggested that the reason why the elements do not unite is because there is a lack of affinity in the plants crossed. But an explanation of this sort does not explain, for we hesitate to define lack of affinity. It is well to refer here to the statements that closeness of relationship marks the limit of successful grafting. And the same thing may be said in regard to the limits of crossing. As a rule, only closely related plants can be crossed. Affinity, resemblance, or likeness, allows the union of similar parts. Dissimilarity or unlikeness prohibits union. Now, if we return to reciprocal crosses, we find it difficult to make the application. How is it that the golden custard squash is so like the crookneck that it will receive its pollen and bear seed; but the crookneck be so unlike the golden custard as to refuse to be fertilized by such pollen? If such conditions exist, they must be centered in the sexual elements, the gametes.

In speaking of the delicate adjustment of plants, Wallace[1] has suggested that the reason certain plants refuse to cross reciprocally, is an indication of disturbance of the sexual organs. Very likely there is a germ of truth in this suggestion, and we might add that the sexual organs of impossible reciprocals are in a state of unstable equilibrium. This seems rational from the nature of the case; but what is disturbed? Is it the molecular swing of the basis of life? Is some constitutional character disturbed? Or is there a disturbance in one or both cases? This feature of the problem must remain as a matter of conjecture.

Since there does not appear to be a difference in structure of the gametes, it is at least possible that a disturbance has taken

1. Alfred R. Wallace, Darwinism, page 155.

place which has resulted in some chemical differentiation. If this theory is tenable, fusion is prohibited because the chemical stimulus which unites the elements is impaired or destroyed. Some slight change in the chemical composition of the gametes or of the embryo-sac, perhaps both, would disturb the equilibrium and impair the forces which cause fusion. As it now appears, the egg-cell of crookneck is indisposed toward pollen spores of golden custard, that is, there is a manifest expression of selection with crookneck pistils. Not all pollen is congenial, some pollen spores will not be received, and among these non-receptive gametes are to be included those of golden custard. In other words, this is apparently an example of what has been called "sexual elective affinity."

(3.) Some products of the crosses.

When the fruits mentioned above had come to full maturity they were cut open and in some cases many seeds were found which appeared to be well formed. Such results were surprising, but were not to be accepted as demonstrations that these reciprocal crosses can be made, until the fertility of the seeds had been determined and the character of the offspring observed.

The seeds were dried and planted to determine if they were viable; and if so, to ascertain if the offspring were also fertile. The seeds were planted in the greenhouse, and the following is the record of their germination.

No. of the fruit.	No. of seed produced.	No. of seeds planted.	No. of seeds germinated.	Per cent germinated.
1	195	25	5	20
2	160	25	0	0
3	33	25	5	20
4	40	25	5	20
5	37	25	15	60
6	56	25	14	56

The higher percentage of germination in the last two cases may be accounted for by the more careful selection of seed; only the best were planted. The above figures are of value in showing the degree of viability of the seeds.

Bringing forward the figures previously used we have the following statement: Of 284 pollinations more than 200 pistils were left on the vines as long as they remained intact. From

these 200 pistils 18 well formed fruits were obtained; of the 18 fruits only 6 produced seeds with embryos, and from these 6 only 5 contained fertile seeds. Germinative tests of these seeds showed on the average that 30 per cent were viable. These facts emphasize the importance of a practical study of the obscure problems concerning plant development as opposed to generalizations from a study of the minute structure of the organs.

From the foregoing results the following statements seem reasonable:

I. Reciprocal crosses with crookneck and golden custard squashes are not absolutely impossible.

II. Golden custard pollen varies in its degree of potency and shows all possible stages, ranging from perfect fertility to perfect sterility. These stages are shown in the decay of crookneck pistils, when pollinated with golden custard; in microscopical sections of such pistils; in the partial and complete development of fruit and seed; and in the sterility and fertility of seed.

GENERAL SUMMARY.

From the foregoing investigations the following conclusions seem justifiable:

Failure in making reciprocal crosses may be explained by some incompatibility in the structure of the flowers. Such barriers may result from delicate structures, and constitute mechanical difficulties.

Failure of pollen to produce fecundation may be explained by the incomplete development of pollen tubes, or, in cases of complete development, by the non-fusion of nuclei.

Failure in the fusion of male and female nuclei is indicative of disturbance of the sexual organs. Such disturbances may be chemical, but are probably constitutional in nature.

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DESCRIPTION OF PLATES.

Figure 10. Male blossom of squash tied to insure purity of pollen. See page 91.

Figure 11. Female blossom of squash (Crookneck) previous to pollination; tied to prevent accidental crossing. See page 91.

Figure 12. Longitudinal section of Crookneck and Golden Custard squashes, at time of pollination.

Figure 13. Crookneck crossed by Golden Custard, at maturity. No. 2 mentioned on page 96.

Figure 14. Crookneck crossed by Golden Custard, at maturity. No. 4 mentioned on page 96.

Figure 15. Crookneck crossed by Golden Custard, 14 days after pollination. No ovules were fertilized and the fruit had begun to decay.

Figure 16. The offspring of Crookneck crossed by Golden Custard. Note the intermediate characters.

Figure 17. Golden Bush crossed by Hubbard. Illustrating the possible stimulating effect of pollen upon the ovary in the absence of fertilization. No fertile seeds were produced.

Figure 18. Stigmas of Red Currant and Yellow Plum tomatoes. See page 89.

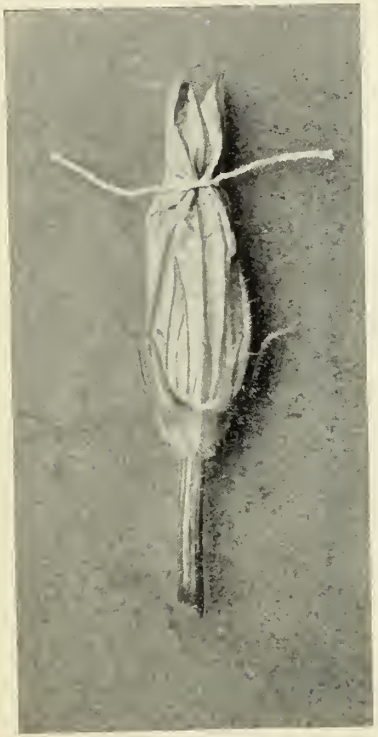

Figure 10.

Figure 11.

Figure 12.

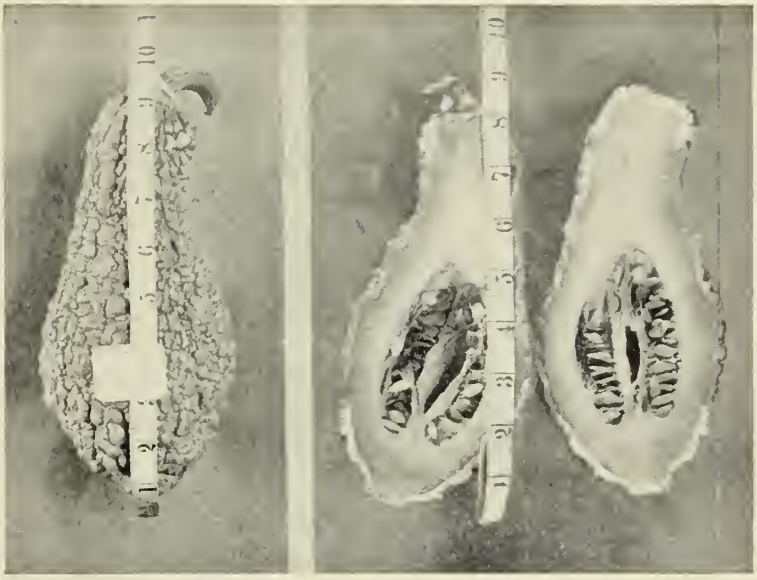

Figure 13.

Figure 14.

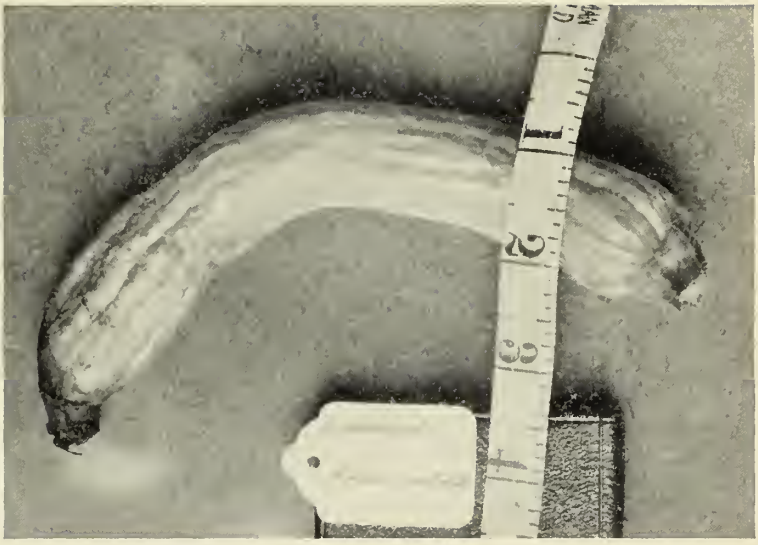

Figure 15.

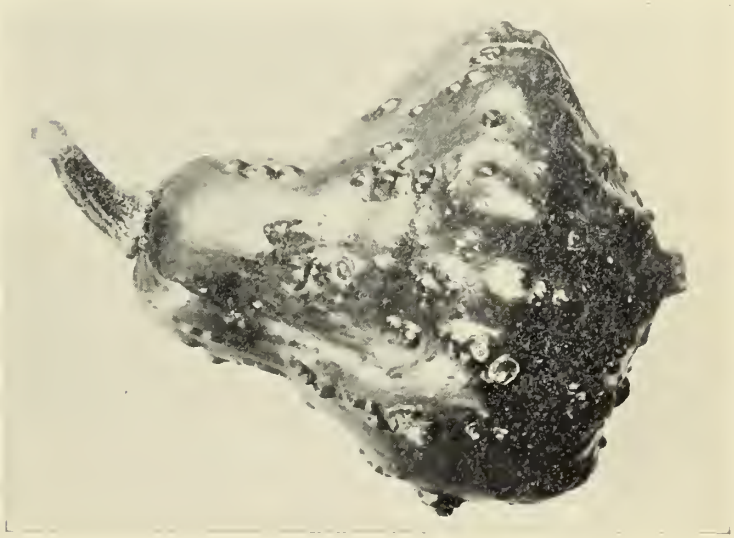

Figure 16.

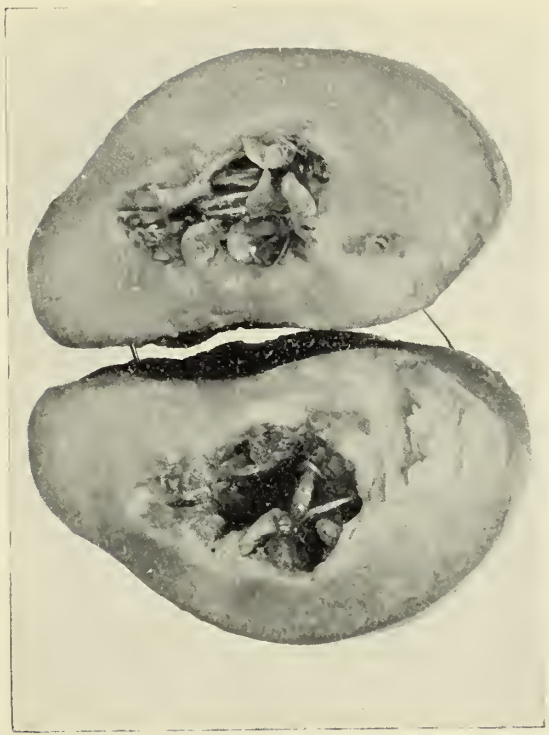

Figure 17.

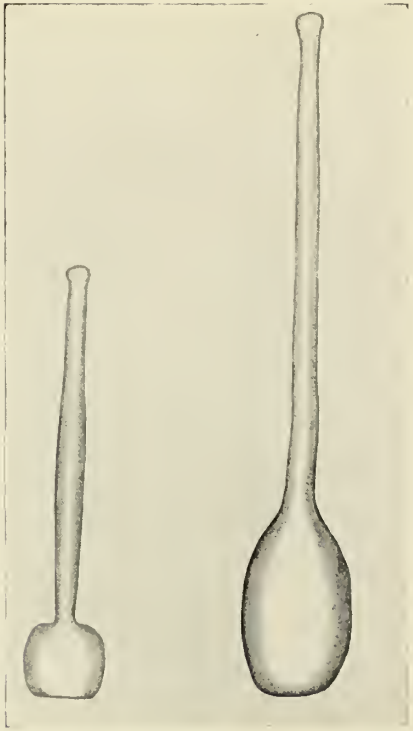

Figure 18.